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=> d his

     (FILE 'HOME' ENTERED AT 11:31:39 ON 20 MAR 2003)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:32:00 ON 20 MAR 2003
L1         47353 S ALPHA (A) AMYLASE?
L2        275104 S FUNGAMYL OR FUNGAL
L3          1373 S L1 AND L2
L4         77681 S THERMOSTAB? OR ACID (A) RESISTANT
L5            66 S L3 AND L4
L6       1495798 S MUTANT? OR VARIANT?

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:47:22 ON 20 MAR 2003
L7         47353 S ALPHA (A)AMYLASE?
L8        275104 S FUNGAL OR FUNGAMYL
L9          1373 S L7 AND L8
L10            6 S L5 AND L6
L11            6 DUP REM L10 (0 DUPLICATES REMOVED)
L12          322 S SVENDSEN A/AU
                 E SVENDSEN A/AU
L13          322 S E3
                 E BISGARD-FRANTZEN/AU
L14            2 S E4
                 E PEDERSEN S/AU
L15         1280 S E3
L16         1602 S L13-L15
L17            6 S L3 AND L16
L18            3 DUP REM L17 (3 DUPLICATES REMOVED)
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PASSWORD:
 * * * * * * RECONNECTED TO STN INTERNATIONAL * * * * * *
SESSION RESUMED IN FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' AT 11:46:33 ON 20 MAR 2003
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   3 FILES SEARCHED...
   4 FILES SEARCHED...
L6   1495798 MUTANT? OR VARIANT?

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	14.63	14.84

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	14.63	14.84

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=> s alpha (a)amylase?
L7        47353 ALPHA (A) AMYLASE?

=> s fungal or fungamyl
<------User Break------>

SEARCH ENDED BY USER

=> s fungal or fungamyl
L8       275104 FUNGAL OR FUNGAMYL

=> s l7 and l8
L9         1373 L7 AND L8

=> d his

     (FILE 'HOME' ENTERED AT 11:31:39 ON 20 MAR 2003)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:32:00 ON 20 MAR 2003
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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:47:22 ON 20 MAR 2003
L7         47353 S ALPHA (A)AMYLASE?
L8        275104 S FUNGAL OR FUNGAMYL
L9          1373 S L7 AND L8

=> s l5 and l6
L10            6 L5 AND L6

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11            6 DUP REM L10 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L11   ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290  BIOTECHDS
TITLE:            New variant of Fungamyl-like
                  alpha-amylase, useful for production of
                  maltose syrups, includes mutations that improve stability
                  against heat and acidic pH;
                     plasmid pTAKA17 expression in bacterium cell for syrup
                     production, dough improvement, brewing and starch
                     liquefaction
AUTHOR:           Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE:  Novozymes
LOCATION:         Bagsvaerd, Denmark.
PATENT INFO:      WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO:    DK 1999-1617 10 Nov 1999
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2001-367478 [38]
AB    A variant (A) of a Fungamyl-like alpha-
      amylase (EC-3.2.1.1) is claimed.  (A) has alteration in one of
```

the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) **variants** of **Fungamyl**-like enzymes with increased **thermostability**; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L11 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2003 ISI (R)
ACCESSION NUMBER: 1998:302504 SCISEARCH
THE GENUINE ARTICLE: ZG858
TITLE: Characterization of a **fungal** amylase from Mucor sp. associated with the marine sponge Spirastrella sp.
AUTHOR: Mohapatra B R; Banerjee U C (Reprint); Bapuji M
CORPORATE SOURCE: INST MICROBIAL TECHNOL, BIOCHEM ENGN RES & PROCESS DEV CTR, SECTOR 39A, CHANDIGARH 160036, INDIA (Reprint); INST MICROBIAL TECHNOL, BIOCHEM ENGN RES & PROCESS DEV CTR, CHANDIGARH 160036, INDIA; REG RES LAB, CSIR, FOREST & MARINE PROD DIV, BHUBANESWAR 751013, ORISSA, INDIA
COUNTRY OF AUTHOR: INDIA
SOURCE: JOURNAL OF BIOTECHNOLOGY, (5 FEB 1998) Vol. 60, No. 1-2, pp. 113-117.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0168-1656.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 21
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A novel amylase was isolated from the Mucor sp. associated with the marine sponge Spirastrella sp., grown at 30 degrees C. The enzyme has an optimum pH of 5.0 and an optimum temperature of 60 degrees C. The half lives of the partially purified enzyme at 55 and 60 degrees C were 120 and 50 min, respectively. The activation and deactivation energies of the partially purified enzyme were 46.60 and 157.05 kJ mol(-1), respectively. The enzyme activity was not affected by the addition of 3% NaCl, 10 mM Ca2+ and 25 mM Mg2+, but was strongly inhibited by EDTA.

L11 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1997-08349 BIOTECHDS
TITLE: Combined desizing and stone-washing of dyed denim; using **alpha-amylase**, cellulase, endo-glucanase and optionally a **thermostable** lipase
AUTHOR: Lund H
PATENT ASSIGNEE: Novo-Nordisk
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 9718286 22 May 1997
APPLICATION INFO: WO 1996-DK469 15 Nov 1996
PRIORITY INFO: DK 1995-1278 15 Nov 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-289265 [26]
AB A one step process for combining desizing and stone-washing of dyed denim

is claimed, in which the denim is treated with an amylolytic enzyme in combination with a first abrading monocomponent endo-glucanase and a second streak-reducing endo-glucanase. Preferably, the amylolytic enzyme is an **alpha-amylase** (EC-3.2.1.1) from Bacillus sp. (especially Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis or Bacillus stearothermophilus) or by Aspergillus sp., or an oxidation-stable **alpha-amylase mutant**. The first endo-glucanase is a **fungal** EG-V cellulase (EC-3.2.1.4), especially from Humicola insolens DSM 1800 (protein sequence specified), Fusarium oxysporum DSM 2672 or Myceliophthora thermophila CBS 117.65, or a **fungal** EG-III cellulase from Trichoderma sp. The second endo-glucanase may be derived from Humicola (especially Humicola insolens (protein sequence specified)), Trichoderma sp. (especially Trichoderma reesei), Myceliophthora sp., Penicillium sp., Irpex sp., Aspergillus sp., Scytalidium sp. or Fusarium sp. (especially Fusarium oxysporum) A **thermostable** lipase (EC-3.1.1.3) derived from Pseudomonas sp. or Candida sp. may be included. (31pp)

L11 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 96433120 MEDLINE
DOCUMENT NUMBER: 96433120 PubMed ID: 8836148
TITLE: Raw-starch-digesting and **thermostable** **alpha-amylase** from the yeast Cryptococcus sp. S-2: purification, characterization, cloning and sequencing.
AUTHOR: Iefuji H; Chino M; Kato M; Iimura Y
CORPORATE SOURCE: National Research Institute of Brewing, Higashi-Hiroshima, Japan.
SOURCE: BIOCHEMICAL JOURNAL, (1996 Sep 15) 318 (Pt 3) 989-96. Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-D83540; GENBANK-D83541
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961120

AB A starch-degrading enzyme produced by the yeast Cryptococcus sp. S-2 was purified in only one step by using an alpha-cyclodextrin-Sepharose 6B column, and was characterized as an **alpha-amylase** (EC 3.2.1.1). The molecular mass and isoelectric point of purified **alpha-amylase** (AMY-CS2) were estimated to be 66 kDa and 4.2 respectively. AMY-CS2 has raw-starch-digesting and raw-starch-absorbing activities. Furthermore it was shown to be **thermostable**. An open reading frame of the cDNA specified 611 amino acids, including a putative signal peptide of 20 amino acids. The N-terminal region of AMY-CS2 (from the N-terminus to position 496) had 49.7% similarity with the whole region of **alpha-amylase** from Aspergillus oryzae (Taka-amylase), whereas the C-terminal region had a sequence that was similar to the C-terminal region of glucoamylase G1 from A. niger. In addition, putative raw-starch-binding motifs exist in some amylolytic enzymes. A **mutant** AMY-CS2 that lacks the C-terminal domain lost not only its ability to bind or digest raw starch, but also its **thermostability**. Consequently it is possible that the putative raw-starch-binding domain of AMY-CS2 plays a role not only in the molecule's raw-starch-digesting ability but also in its **thermostability**.

L11 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1990-14946 BIOTECHDS
TITLE: Recent results of amylolytic enzymes research; Aspergillus niger and Bacillus licheniformis strain improvement for **thermostable alpha-amylase** and glucoamylase production; UV-irradiation mutagenesis, selection, transformation or protoplast fusion
AUTHOR: Hoschke A
LOCATION: Food Biotechnology Division of C.F.R.I., Hungary.
SOURCE: Acta Aliment.Acad.Sci.Hung.; (1990) 19, 2, 210-11
CODEN: AAASCO
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recent results of strain and culture improvement for increasing amylolytic enzyme production in Hungary were described. A multi-step combined strain improvement technique was used to increase **thermostable alpha-amylase** (EC-3.2.1.1) production by Bacillus licheniformis ATCC 27811. A 20-fold increase in enzyme production was achieved by multi-step UV irradiation mutagenesis and selective isolation. Strain improvement was also performed by genetic engineering. 150 **Fungal** strains isolated for glucoamylase (EC-3.2.1.3) production included a maize (Zea mays) isolate, KEKI P-36, and an induced **mutant** of Aspergillus niger (ATCC 22343). Multi-step mutagenesis of KEKI P-36 increased enzyme production 25-fold. Protoplast fusion of A. niger (ATCC 22343) yielded strains with low productivity. Gene bank construction facilitated isolation of glucoamylase genes. A. niger ATCC 22343/65 was grown in 10 l fermentors and the changes in glucoamylase, transglucosidase and **alpha-amylase** activities, mycelium mass, pH and glucose content were measured during fermentation. The maximal glucoamylase yield was 18-20 GAU/ml. (0 ref)

L11 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1980:490840 HCAPLUS
DOCUMENT NUMBER: 93:90840
TITLE: Purification methods and properties of **fungal** and bacterial amylases
AUTHOR(S): Kvesitadze, G. I.
CORPORATE SOURCE: Inst. Biokhim. Rast., Tbilisi, USSR
SOURCE: Metody Poluch. Vysokoochishchennykh Fermentov (1978), 125-8. Editor(s): Glemzha, A. A. Vses. Nauchno-Issled. Inst. Prikl. Enzimol.: Vilnius, USSR.
CODEN: 43SAAT
DOCUMENT TYPE: Conference
LANGUAGE: Russian

AB The acid-stable and acid-labile .**alpha**.-**amylases** from **mutant** strains of Aspergillus oryzae and A. niger and .**alpha**.-**amylase** from Bacillus subtilis were purified by a method involving 2-3 operations which gave >80% yield. The prepns. were homogeneous as judged by SDS-polyacrylamide gel electrophoresis and the mol. wts. of acid-stable and acid-labile **fungal** amylases were 58,000 and 51,000, resp. The N-terminal amino acid was alanine for the acid-labile and leucine for the acid-stable enzyme, and the C-terminal amino acids were serine and valine, resp. Ca2+ was found to play an important role in the stabilization of enzyme. The **fungal** amylases contained 1 SH group which bound with Ca2+; this interaction was important in maintaining the catalytically active conformation. The SH group was chem. modified only after removal of Ca2+ by EDTA or other chelators. Amylase of B. subtilis was more **thermostable** than the **fungal** amylases. The **thermostability** of the acid-stable enzyme of A. niger at 50-60.degree. was an order of magnitude higher than that of the enzyme from A. oryzae.

```
=> s svendsen a/au
L12         322 SVENDSEN A/AU

=> e svendsen a/au
E1            1      SVENDSE F/AU
E2            6      SVENDSEN/AU
E3          322 --> SVENDSEN A/AU
E4          358      SVENDSEN A B/AU
E5          109      SVENDSEN A BAERHEIM/AU
E6            1      SVENDSEN A BARHEIM/AU
E7           12      SVENDSEN A J/AU
E8            8      SVENDSEN A K/AU
E9            4      SVENDSEN A M/AU
E10           2      SVENDSEN A M B/AU
E11           6      SVENDSEN AA P/AU
E12           3      SVENDSEN AGNER/AU

=> s e3
L13         322 "SVENDSEN A"/AU

=> e bisgard-frantzen/au
E1            1      BISGARD P/AU
E2            1      BISGARD POUL/AU
E3            0 --> BISGARD-FRANTZEN/AU
E4            2      BISGARDFRANTZEN H/AU
E5            1      BISGAWA F/AU
E6            2      BISGAY K/AU
E7            1      BISGAY L/AU
E8            6      BISGEIER G/AU
E9            8      BISGEIER G P/AU
E10           1      BISGEIER GEORGE/AU
E11           2      BISGES A/AU
E12          13      BISGES A D/AU

=> s e4
L14           2 "BISGARDFRANTZEN H"/AU

=> e pedersen s/au
E1            1      PEDERSEN ROY MARTIN/AU
E2            5      PEDERSEN RUDI/AU
E3         1280 --> PEDERSEN S/AU
E4            4      PEDERSEN S */AU
E5          542      PEDERSEN S A/AU
E6            4      PEDERSEN S A S/AU
E7            1      PEDERSEN S ANKER/AU
E8          330      PEDERSEN S B/AU
E9            1      PEDERSEN S BOEL/AU
E10          55      PEDERSEN S C/AU
E11          12      PEDERSEN S D/AU
E12         174      PEDERSEN S E/AU

=> s e3
L15        1280 "PEDERSEN S"/AU

=> d his

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
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L5            66 S L3 AND L4
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L8        275104 S FUNGAL OR FUNGAMYL
L9          1373 S L7 AND L8
L10            6 S L5 AND L6
L11            6 DUP REM L10 (0 DUPLICATES REMOVED)
L12          322 S SVENDSEN A/AU
                 E SVENDSEN A/AU
L13          322 S E3
                 E BISGARD-FRANTZEN/AU
L14            2 S E4
                 E PEDERSEN S/AU
L15         1280 S E3

=> s l13-l15
L16        1602 (L13 OR L14 OR L15)

=> s l3 and l16
L17           6 L3 AND L16

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18           3 DUP REM L17 (3 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L18   ANSWER 1 OF 3  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-04043  BIOTECHDS
TITLE:            Production of fermentation product, e.g. ethanol, involves
                  carrying out a fermentation step with at least one
                  carbohydrate-source generating enzyme activity and at least
                  one alpha-amylase activity;
                     with use of Bacillus stearothermophilus, Aspergillus
                     niger, Talaromyces emersonii or Rhizomucor miehei
                     alpha-amylase
AUTHOR:           OLSEN H S; PEDERSEN S; BECKERICH R; VEIT C; FELBY C
PATENT ASSIGNEE:  NOVOZYMES AS; NOVOZYMES NORTH AMERICA INC
PATENT INFO:      WO 2002074895 26 Sep 2002
APPLICATION INFO: WO 2002-DK179 19 Mar 2002
PRIORITY INFO:    US 2001-304380 10 Jul 2001; US 2001-277383 19 Mar 2001
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2002-723447 [78]
AB    DERWENT ABSTRACT:
      NOVELTY - A fermentation product is produced by carrying out a
      fermentation step in the presence of at least one carbohydrate-source
      generating enzyme activity and at least one alpha-
      amylase activity.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the
      following: (a) A composition comprising: (i) carbohydrate-source
      generating enzyme activity; (ii) alpha-amylase
      activity, protease activity; and (iii) debranching enzyme activity; and
      (b) Use of the composition for saccharification and/or fermentation, for
      ethanol production or for beer or wine production.
```

BIOTECHNOLOGY - Preferred Component: The carbohydrate-source generating enzyme is a glucoamylase particularly derived from Aspergillus niger or Talaromyces emersonii, beta-amylase particularly derived from barley or a maltogenic amylase particularly derived from Bacillus stearothermophilus. The **alpha-amylase** is an acid **alpha-amylase**, particularly an acid **fungal alpha-amylase**, e.g. an acid **fungal alpha-amylase** derived from A. niger or A. oryzae. The ratio between the acid **fungal alpha-amylase** activity (AFAU) per glucoamylase activity (AGU) (AFAU per AU) is at least0.1 (particularly at least0.16), preferably 0.12-0.3. The protease is an acid protease, particularly an acid **fungal** protease, e.g. an acid **fungal** protease derived from a strain of Aspergillus (particularly A. niger or A. oryzae) or a strain of Rhizomucor (particularly R. miehei) or a bacterial protease, e.g. acid, neutral or alkaline protease, (e.g., a protease derived from a strain of Bacillus) particularly ALCALASE or NEUTRASE. The debranching enzyme is an isoamylase (E.C. 3.2.1.68) or pullulanase (E.C. 3.2.1.41), particularly a pullulanase derived from Bacillus sp., e.g. a strain of B. deramificans, B. acidopullulyticus or B. naganoensis. The glucoamylase/pullulanase ratio determined as AGU/PUN is 5:1-1:5. The micro-organism is a yeast, e.g. a yeast belonging to Saccharomyces spp. (particularly Saccharomyces cerevisae). The material to be fermented is a liquefied whole grain mash or a side stream from starch processing, particularly liquefied starch with a DE of 8-10. The yeast cell wall degrading enzyme is a preparation, e.g. the product GLUCANEX (RTM: enzyme) derived from Trichoderma harzianum.

USE - The inventive process is used for producing a fermentation product, preferably ethanol, beer or wine. The produced ethanol can be used as fuel ethanol, drinking ethanol (i.e., potable neutral spirits) or industrial alcohol. The grain, a left-over from the fermentation or distillation steps, is typically used for animal feed either in liquid or dried form.

ADVANTAGE - The inventive method results in increased fermentation rate and ethanol yield.

EXAMPLE - Washed yeast (2.5 g) was suspended in ion-exchanged water (100 mL) at room temperature. The suspension was stirred on a magnetic stirrer for 15 minutes. Samples (15 mL) were transferred to centrifuge tubes with volume indication. Sodium chloride (NaCl), calcium chloride (CaCl2) and Rhizomucor miehei protease was added to create the solutions of 250 mM NaCl, 4 mM calcium chloride (CaCl2) and 4 mM CaCl2 and R. miehei protease. Incubation of the solutions was made at room temperature for 15 minutes in a rotary shaker, which turned the closed tubes end-over-end at 20 rpm. The tubes were left in vertical position for 60 minutes after which the volume of the sediment was measured. The results showed the effect of R. miehei protease on volume of sediment. For the solution containing 250 mM NaCl the volume of sediment was 0.165 mL, and for the solution containing 4 mM CaCl2 the volume of sediment was 0.245 mL and for the solution containing 4 mM CaCl2 and R. miehei protease the volume of sediment was 0.194 mL.(38 pages)

L18 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of **Fungamyl**-like **alpha-amylase**, useful for production of maltose syrups, includes mutations that improve stability against heat and acidic pH;
plasmid pTAKA17 expression in bacterium cell for syrup production, dough improvement, brewing and starch liquefaction
AUTHOR: Bisgard-Frantzen H; **SvendSen A; Pedersen S**
PATENT ASSIGNEE: Novozymes

LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]

AB A variant (A) of a **Fungamyl**-like **alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of **Fungamyl**-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L18 ANSWER 3 OF 3 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000417503 MEDLINE
DOCUMENT NUMBER: 20384196 PubMed ID: 10924103
TITLE: Structural analysis of a chimeric bacterial **alpha-amylase**. High-resolution analysis of native and ligand complexes.
AUTHOR: Brzozowski A M; Lawson D M; Turkenburg J P; Bisgaard-Frantzen H; **Svendsen A**; Borchert T V; Dauter Z; Wilson K S; Davies G J
CORPORATE SOURCE: Department of Chemistry, Structural Biology Laboratory, University of York, Heslington, UK.
SOURCE: BIOCHEMISTRY, (2000 Aug 8) 39 (31) 9099-107. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1E3X; PDB-1E3Z; PDB-1E43; PDB-1E4PHI
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000915
Last Updated on STN: 20000922
Entered Medline: 20000907

AB Several chimeric **alpha-amylases** genes were constructed by an in vivo recombination technique from the Bacillus amyloliquefaciens and Bacillus licheniformis genes. One of the fusion amylases (hereafter BA2), consisting of residues 1-300 from B. amyloliquefaciens and 301-483 from B. licheniformis, has been extensively studied by X-ray crystallography at resolutions between 2.2 and 1.7 A. The 3-dimensional structure of the native enzyme was solved by multiple isomorphous replacement, and refined at a resolution of 1.7 A. It consists of 483 amino acids, organized similarly to the known B. lichiniformis **alpha-amylase** structure [Machius et al. (1995) J. Mol. Biol. 246, 545-559], but features 4 bound calcium ions. Two of these form part of a linear cluster of three ions, the central ion being attributed to sodium. This cluster lies at the junction of the A and B domains with one calcium of the cluster structurally equivalent to the major Ca(2+) binding site of **fungal alpha-amylases**. The third calcium ion is found at the interface of the A and C domains. BA2 contains a fourth calcium site, not observed in the B. licheniformis

alpha-amylase structure. It is found on the C domain where it bridges the two beta-sheets. Three acid residues (Glu261, Asp328, and Asp231) form an active site similar to that seen in other amylases. In the presence of TRIS buffer, a single molecule of TRIS occupies the -1 subsite of the enzyme where it is coordinated by the three active-center carboxylates. Kinetic data reveal that BA2 displays properties intermediate to those of its parents. Data for crystals soaked in maltooligosaccharides reveal the presence of a maltotriose binding site on the N-terminal face of the (beta/alpha)(8) barrel of the molecule, not previously described for any **alpha-amylase** structure, the biological function of which is unclear. Data for a complex soaked with the tetrasaccharide inhibitor acarbose, at 1.9 A, reveal a decasaccharide moiety, spanning the -7 to +3 subsites of the enzyme. The unambiguous presence of three unsaturated rings in the (2)H(3) half-chair/(2)E envelope conformation, adjacent to three 6-deoxypyranose units, clearly demonstrates synthesis of this acarbose-derived decasaccharide by a two-step transglycosylation mechanism.

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     (FILE 'HOME' ENTERED AT 11:31:39 ON 20 MAR 2003)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:32:00 ON 20 MAR 2003
L1         47353 S ALPHA (A) AMYLASE?
L2        275104 S FUNGAMYL OR FUNGAL
L3          1373 S L1 AND L2
L4         77681 S THERMOSTAB? OR ACID (A) RESISTANT
L5            66 S L3 AND L4
L6       1495798 S MUTANT? OR VARIANT?

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:47:22 ON 20 MAR 2003
L7         47353 S ALPHA (A)AMYLASE?
L8        275104 S FUNGAL OR FUNGAMYL
L9          1373 S L7 AND L8
L10            6 S L5 AND L6
L11            6 DUP REM L10 (0 DUPLICATES REMOVED)
L12          322 S SVENDSEN A/AU
                 E SVENDSEN A/AU
L13          322 S E3
                 E BISGARD-FRANTZEN/AU
L14            2 S E4
                 E PEDERSEN S/AU
L15         1280 S E3
L16         1602 S L13-L15
L17            6 S L3 AND L16
L18            3 DUP REM L17 (3 DUPLICATES REMOVED)

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	~~Issue Date~~	Pages	Document ID	Title
1	20021107	23	US 20020164723 A1	Method of producing saccharide preparations
2	20020827	99	US 6440716 B1	.alpha.-amylase mutants
3	20020516	7	US 20020058086 A1	Methods and compositions for retarding the staling of baked goods
4	20011211	12	US 6329182 B1	Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups
5	20011016	19	US 6303346 B1	Method of producing saccharide preparations
6	20001024	15	US 6136571 A	Method of producing saccharide preparations
7	20001010	19	US 6129788 A	Method of producing saccharide preparations
8	20000215	14	US 6025168 A	Method for the production of isomalto-oligosaccharide rich syrups
9	20000208	100	US 6022724 A	.alpha.-amylase mutants
10	19991123	100	US 5989169 A	.alpha.-amylase mutants

	~~Issue Date~~	Pages	Document ID	Title
11	19950613	7	US 5424299 A	Composition and method for rejuvenating enteral feeding tubes
12	19900918	8	US 4957563 A	Starch conversion
13	19880105	11	US 4717662 A	Thermal stabilization of alpha-amylase
14	19851224	15	US 4560651 A	Debranching enzyme product, preparation and use thereof
15	19831122	18	US 4416903 A	Antistaling baking composition
16	19820316	13	US 4320151 A	Antistaling baking composition
17	19770920	6	US 4049466 A	Levulose containing sweetening compositions

	~~Issue Date~~	Pages	Document ID	Title
1	20021107	23	US 20020164723 A1	Method of producing saccharide preparations
2	20020827	99	US 6440716 B1	.alpha.-amylase mutants
3	20011016	19	US 6303346 B1	Method of producing saccharide preparations
4	20001024	15	US 6136571 A	Method of producing saccharide preparations
5	20001010	19	US 6129788 A	Method of producing saccharide preparations
6	20000208	100	US 6022724 A	.alpha.-amylase mutants
7	19991123	100	US 5989169 A	.alpha.-amylase mutants

	~~Issue Date~~	Pages	Document ID	Title
1	20030306	37	US 20030044954 A1	Alpha-amylase variants
2	20030304	63	US 6528298 B1	.alpha.-amylase mutants
3	20021219	137	US 20020192792 A1	Laccase mutants
4	20021217	120	US 6495357 B1	Lipolytic enzymes
5	20021119	27	US 6482622 B1	Amylolytic enzyme variants
6	20021107	23	US 20020164723 A1	Method of producing saccharide preparations
7	20020910	26	US 6448049 B1	Starch conversion process
8	20020905	17	US 20020123123 A1	Cutinase variants
9	20020827	99	US 6440716 B1	.alpha.-amylase mutants
10	20020820	46	US 6436888 B1	.alpha.-amylase mutants
11	20020725	44	US 20020098996 A1	Amylase variants
12	20020627	63	US 20020081670 A1	Starch debranching enzymes
13	20020625	34	US 6410295 B1	Alpha-amylase variants

	~~Issue Date~~	Pages	Document ID	Title
14	20020606	37	US 20020068352 A1	Alpha-amylase variants with altered 1, 6-activity
15	20020416	14	US 6372465 B1	Haloperoxidases with altered pH profiles
16	20020326	64	US 6361989 B1	.alpha.-amylase and .alpha.-amylase variants
17	20020305	43	US 6352851 B1	Glucoamylase variants
18	20020226	82	US 6350599 B1	Pullulanase variants and methods for preparing such variants with predetermined properties
19	20011211	23	US 6329186 B1	Glucoamylases with N-terminal extensions
20	20011115	15	US 20010041666 A1	Haloperoxidases with altered pH profiles
21	20011108		US 20010039253 A1	Alpha-amylase mutants
22	20011018		US 20010031490 A1	Laccase mutants
23	20011016	19	US 6303346 B1	Method of producing saccharide preparations
24	20011002		US 6297038 B1	Amylase variants
25	20010821		US 6277611 B1	Laccase mutants
26	20010724		US 6265197 B1	Starch debranching enzymes
27	20010424		US 6221821 B1	Haloperoxidases with altered pH profiles
28	20010417		US 6218170 B1	Laccase mutants

	~~Issue Date~~	Pages	Document ID	Title
29	20010320		US 6204232 B1	.alpha.-amlase mutants
30	20010306		US 6197565 B1	.alpha.-Amylase variants
31	20010213		US 6187576 B1	.alpha.-amylase mutants
32	20010206		US 6184015 B1	Laccase mutants
33	20001219		US 6162628 A	Maltogenic alpha-amylase variants
34	20001107		US 6143708 A	.alpha.-amylase mutants
35	20001031		US 6140092 A	Laccase mutants
36	20001024	15	US 6136571 A	Method of producing saccharide preparations
37	20001010	19	US 6129788 A	Method of producing saccharide preparations
38	20000725		US 6093562 A	Amylase variants

	~~Issue Date~~	Pages	Document ID	Title
39	20000711		US 6087149 A	Starch conversion process
40	20000613	21	US 6074863 A	C. antarctica lipase variants
41	20000509		US 6060442 A	Laccase mutants
42	20000208	100	US 6022724 A	.alpha.-amylase mutants
43	20000201	20	US 6020180 A	C. antarctica lipase and lipase variants
44	19991207		US 5998353 A	Laccase mutants
45	19991123	100	US 5989169 A	.alpha.-amylase mutants
46	19991116		US 5985818 A	Laccase mutants

	~~Issue Date~~	Pages	Document ID	Title
47	19991102		US 5976855 A	Method of preparing a variant of a lipolytic enzyme
48	19991019		US 5968883 A	Peroxidase variants
49	19990720		US 5925554 A	Myceliophthora and scytalidium laccase variants
50	19990406		US 5892013 A	Lipase variants
51	19990209		US 5869438 A	Lipase variants
52	19990202		US 5866526 A	Enzyme preparation comprising a modified enzyme
53	19981103		US 5830837 A	Amylase variants

	~~Issue Date~~	Pages	Document ID	Title
54	19981006	26	US 5817495 A	H.sub.2 O.sub.2 -stable peroxidase variants
55	19980901		US 5801043 A	Amylase variants
56	19980519	50	US 5753460 A	Amylase variants
57	19971007		US 5674833 A	Detergent compositions containing protease and novel inhibitors for use therein

	~~Issue Date~~	Pages	Document ID	Title
1	20021107	23	US 20020164723 A1	Method of producing saccharide preparations
2	20011211	12	US 6329182 B1	Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups
3	20011016	19	US 6303346 B1	Method of producing saccharide preparations
4	20001024	15	US 6136571 A	Method of producing saccharide preparations
5	20001010	19	US 6129788 A	Method of producing saccharide preparations

	L #	Hits	Search Text
1	L1	4928	alpha adj amylase$2
2	L2	27222	fungal or fungamyl
3	L3	570	l1 same l2
4	L4	14634	thermostab$5 or (acid adj resistan$3)
5	L6	149799	mutant$2 or variant$2
6	L7	0	l5 same l6
7	L5	17	l3 same l4
8	L8	243	svendsen.in.
9	L10	48	l3 and l8
10	L11	7	l5 and l8
11	L9	57	l1 and l8
12	L12	1144	pedersen.in.

	L #	Hits	Search Text
13	L13	5	15 and l12